ESRF	**Experiment title:** Fine Structures of Dioxygen Reducing Dinuclear Center (Fea-CuB) of Cytochrome c Oxidase at Oxidized and Oxigenized States	**Experiment number:** LS695
Beamline: ID2	**Date of Experiment:** from: 27-Feb-1997 to: 1-Mar-1997	**Date of Report:** 31-Jul-1997
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Report:

Bovine heart cytochrome c oxidase (COX) is a large mitochondrial inner membrane protein complex which is composed of 13 different subunits with a total molecular weight of 200kDa. It catalyzes the reduction of one molecular oxygen to two water molecules and, coupled to this reaction, protons are pumped across the membrane to produce the transmebrane potential which is required for the synthesis of ATPs in the oxidative phosphorelation in the mitochindrial respiratory chain.

The crystal structure of COX was solved at 2.8 Å resolution[(1)] and possible proton, water and oxygen pathways were also proposed. In order to investigate the structure and function in detail , high resolution data collection was carried out on a beam line ID2 at the ESRF.

X-ray experiment of oxidized COX was carried out under cryo-stream of about 100 K with an image plate detector of MAR. Wave length and camera distance used were 0.998 Å and 270 mm, respectively. 99 flames were obtained by 1 degree oscillation and 4 seconds exposure, and 189 flames by 0.5 degree oscillation and 7 seconds exposure. Only one crystal was used for the whole data collection.

Cell dimensions of the frozen crystal used are a=187.5, b=207.6 and c=178.8 A. The cell dimensions of a and b are shrunk by 1% in comparison with those of unfrosen crystal, which are a=189.1, b=210.5 and c=178.6 Å.

Intensity data were processed by DENZO, and post-refined and scaled by SCALEPAC[2] (Table l). Unfrozen crystals diffract isotropically. However the frozen crystal diffracted anisotropically up to 2.1 Å resolution along one direction and 2.4 Å along another direction. The same frozen crystal diffracted up to 3 Å resolution by using Cu-Ka X-ray of a rotating anode. The merging R-factor and the completeness are 9.4% and 83.6%, respectively, between the resolution range of 200-2.1 Å

Table 1 The statistics of the data processing

Resolution range	200 ~ 2.1 (A)
Observed reflections	4508,489
Independent reflections	365,420
Averaged redundancy	12.3
Completeness	83.6 (%)
Rmerge	9.4 (%)

Riso in F between the frozen crystal and the unfrozen crystal is 15.2 % at 2.8 Aresolution. Crystal structure analysis of the frozen crystal was initiated by the molecular replacement using the oxidized structure solved previously. The initial phases were obtained by the rigid group refinement at 5 Aresolution giving an R-factor of 0.306. The phases were refined and extended gradually to 2.4 Å resolution by the density modification and non-crystallographic two fold symmetry averaging, resulting an R_{free} of 0.289. We built molecular models of whole 26 subunits, metal centers, phospholipids, chelates, decylmaltosides and some unknown hydrocarbons. We have just started structure refinement at 2.4 Å.

References

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